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Prevalence of Merkel Cell Polyomavirus among Swiss Merkel Cell Carcinoma Patients

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Key Words
Carcinogenesis · MCPyV · Merkel cell carcinoma · Polyomavirus

Abstract
Background: Merkel cell carcinoma (MCC) is a malignant neuroendocrine neoplasm which shares structural and immunohistochemical features with neuroectodermally derived cells. One hypothesis claims that it arises from Merkel cells, highly innervated neuroendocrine cells involved in mechanoreception in the skin. The incidence rate of this cancer increases with age and sun exposure as well as after immunosuppression. Recently, the clonal integration of a newly identified polyomavirus called Merkel cell polyomavirus (MCPyV) was reported in up to 80% of MCC tissue. Here we report the incidence rate of MCPyV in MCC patients in Switzerland. ***Methods:*** We performed polymerase chain reaction in a collection of 32 samples obtained from pathology institutes around Switzerland. We used three different published primer sets. As control groups we used 7 squamous cell carcinoma samples and 11 normal skin samples. ***Conclusions:*** We detected viral DNA in 20 out of 30 cases of MCC and in 0 out of 19 control samples. The presence of viral DNA in 66.6% of our MCC tissue specimens confirms the high prevalence of MCPyV in MCC patients described in American, German, French and Hungarian patient collections.

Introduction

Merkel cell carcinoma (MCC) is a rare aggressive neuroendocrine malignancy that frequently shows local recurrence, lymph node and distant metastases. One hypothesis claims that it derives from the normal Merkel cells of the epidermis which are slow-adapting mechanoreceptors. It was first described in 1972 by Toker [1] who identified five patients with unusual skin tumors. Within the last 20 years its incidence has tripled, with an 8% annual increase rate [2], leading the American Cancer society to predict approximately 1,500 new MCC cases in 2008 [3]. It occurs on sun-exposed skin areas in the Caucasian population, predominantly males, with a mean age at the time of diagnosis of 70 years [4]. Its incidence rate rises with immunosuppression, e.g. in organ transplant recipients, as well as in those with chronic lymphocytic leukemia and HIV infection [5–7]. This link to im-

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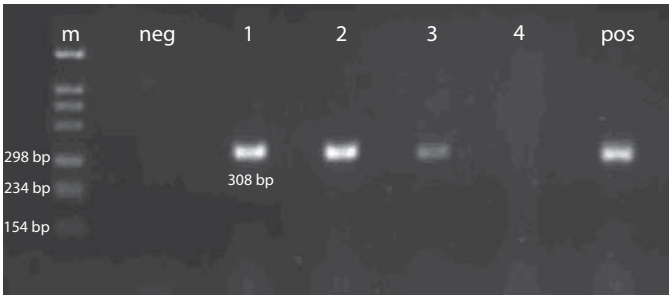

Fig. 1. Representative results of the PCR products (308 bp) with LT3 primers. m = Molecular weight marker VI; neg = negative control (H_2O); 1–4 = MCC samples; pos = positive control (Merkel cell tumor sample obtained from Prof. Axel zur Hausen's lab).

munosuppression raised the question of a possible infectious etiology of this type of cancer.

In 2008, Feng et al. [8] identified a new human polyomavirus, designated as Merkel cell polyomavirus (MCPyV) based on its detection in human MCC samples using a technique known as digital transcriptome subtraction. MCPyV is a double-stranded DNA virus of 5,387 bp that is highly homologous to the murine polyomavirus subgroup [8] and expresses an early region (small and large T antigen) and a late region containing VP1, VP2 and VP3 open reading frames. It has also proved to be monoclonally integrated into the tumor, while the already known polyomaviruses BK, JC, WU and KI [9, 10] have been found to be episomal in the related cancers. This monoclonal pattern of viral integration suggests a possible role of this virus into MCC carcinogenesis. It has also been shown that the MCC harbors genes encoding for a truncated form of large T antigen (LT) mutations, a process believed to be necessary for polyomavirus-mediated carcinogenesis [11].

This study was designed to test the largest number of MCC patients so far studied in Switzerland for the presence of viral DNA using PCR.

Subjects and Methods

Study Design

The study included 32 formalin-fixed paraffin-embedded primary tumor resection specimens from MCC patients obtained from pathology and dermatopathology institutes across Switzerland between 1994 and 2008. Specimens from control groups were also primary tumors. HE and cytokeratin-20 stainings of the selected specimens were repeated to confirm the original diagnosis by a board-certified dermatopathologist (K.K.).

The study was submitted to the ethical committee of Zürich (EK No. 860) and the still alive patients gave informed consent to use their material for the purposes of the study.

DNA Extraction

Five consecutive 5-μm paraffin sections from each specimen were used for DNA extraction. DNA was extracted using a standard procedure protocol. After deparaffinization the tissues were lysed by overnight treatment with proteinase K (56°C). DNA was purified with phenol and was precipitated with 3 M sodium acetate and absolute ethanol. The pellet was extra washed with 70% ethanol to eliminate excess salt. Dried DNA was resuspended in 60 μl of sterile TE and was stored at 4°C before it was used for PCR. Purified DNA was measured using a Nanodrop 1,000 spectrophotometer (Thermoscientific, Wilmington, Del., USA).

Patient Population

The medical records of 9 of the 32 patients included in the study were retrospectively reviewed. The median age of the patients at the time of diagnosis was 77 years; 45 and 55% of the cases were male and female, respectively. One patient had had a squamous cell carcinoma (SCC) 2 years before developing MCC and one patient was diagnosed with melanoma and SCC also 2 years before the diagnosis of MCC. The SCC sample from the last patient was also included in the study.

As control groups, we used 8 SCC and 11 normal skin samples selected from the archives of the Dermatopathology Institute of the University Hospital of Zürich. Control cases were matched with the examined MCC patients only according to age, gender and localization of the primary lesion.

Polymerase Chain Reaction

DNA integrity was tested with β-globin PCR. PCR was performed with 100–120 ng of genomic DNA using the AmpliTaq Gold (Roche Molecular Systems, Basel, Switzerland) in a final volume of 50 μl. For detection of the MCPyV we used 3 primer sets (LT1, LT3 and VP1) as published [8]; two were directed against the LT antigen and one was directed against the VP1 gene. Water was used instead of template DNA as negative control.

Sequence Analysis

PCR products of 8 patients were subjected to sequence analysis. The resulting sequences were compared to the reference sequences of NCBI Entrez Nucleotide Database gb/EU375803 MCPyV isolated from MCC350 using the Blast program [12].

Results

Thirty out of 32 samples revealed a β-globin positive product of 268 bp and these samples were included in the study. In total, 20 out of 30 MCC samples tested positive for the virus with PCR (fig. 1). Five were positive in all primer sets, while in 10 cases no viral DNA was amplifiable. Out of 30 MCC samples, 6 tested positive for the presence of the virus by LT1 (440 bp) primer set, 19 tested positive using the LT3 (308 bp) primer set and 16 tested

positive using the VP1 (351 bp) primer set (fig. 2). Compared with MCPyV DNA-negative cancers, MCPyV DNA-positive cancers tended to be more often located on the extremities (65 vs. 40%, p = 0.193, two-sided χ^2 test), which is in accordance with the findings of Sihto et al. in a set of North European patients [13]. PCR-negative controls remained negative in all experiments.

The virus was not detected in the SCC sample derived from one MCC patient. None of our samples of the control groups (8 SCC and 11 normal skin) were positive for the presence of MCPyV.

The sequenced PCR amplicons showed 96–99% homology to the NCBI Entrez Nucleotide Database gb/EU375803 MCPyV isolated from MCC350. We have not sequenced the entire viral genome for detection of further strain variations.

Clinical follow-ups of the nine patients for whom we received clinical information suggest that there is no significant difference regarding the course of disease in MCPyV-positive and -negative patients, stratified by tumor stage (table 1).

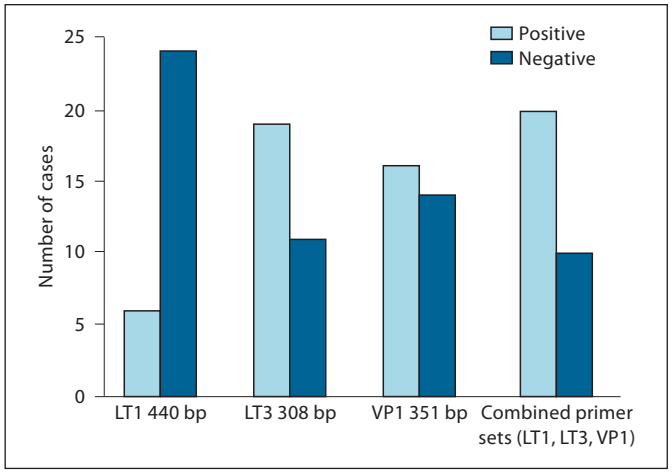

Fig. 2. MCPyV PCR results using the three different primer sets LT1, LT3, VP1, and overall positivity/negativity combining all the results from the three primer sets.

Table 1. Patient characteristics and comparison of tumor stage between MCPyV-positive and -negative patients

Patient ID	Gender	Age at diagnosis	MCPyV	Stage at diagnosis	Stage at date last seen	Follow-up months
1	f	72	neg	stage I	stage II	16.4
2	m	76	neg	stage I	stage III	43.2
3	f	73	neg	stage I	stage I	84
4	m	71	neg	stage I	stage I	77.3
5	f	75	pos	stage I	stage I	32.2
6	f	82	pos	stage I	stage I	16
7	m	83	pos	stage II	stage II	29.4
8	m	79	pos	stage I	stage II	21.2
9	f	84	pos	stage I	stage I	8.33

f = Female; m = male; pos = positive; neg = negative.

Discussion

Even though MCC is one of the most aggressive skin cancers, with a mortality rate of 33% [2], little is known about potential signalling mechanisms that drive carcinogenesis in MCC [14]. The high incidence of the recently described MCPyV in MCC, together with the monoclonal integration pattern [8] and the truncation of LT antigen in MCC [11] shed new light on a potential viral carcinogenesis of MCC.

We demonstrated the presence of viral genome in 66.6% of our cases with PCR, which is similar to the results published from other groups [8, 15–22]. In contrast to Feng et al. [8], we used DNA extracted from formalin-fixed paraffin-embedded specimens. It is known that fixation causes a decrease in amplification efficiency of larger amplicons. Accordingly, we have noticed some differences in detection of MCPyV using the different primer sets. In our lab, LT3 primer proved to be superior for the detection of MCPyV, which is also confirmed by other groups [15–17, 21]. Only 6 out of 19 cases tested positive for the presence of the virus with primer LT1 (440 bp).

We were not able to detect any viral DNA in our control groups, neither in SCC nor in normal skin. Kassem et al. [23] recently demonstrated the presence of viral DNA in 32% of sporadic non-melanoma skin cancers. They also showed that MCPyV was more frequently found in non-melanoma skin cancers of immunosuppressed patients compared to immunocompetent patients. This discrepancy may be due to the higher numbers of cases in their study than in ours.

Andres et al. reported no relevant association of MCPyV with seborrheic keratosis, basal cell carcinoma, and lentigo maligna melanoma in an age- and sex-matched non-MCC population when the virus was found in 6% (2/33) of all the investigated non-MCC skin tumors [24].

In the one patient in our set suffering from a MCPyV-positive MCC and, previously, from an SCC, we did not

find the virus in the SCC. This may be due to a specific targeting of Merkel cells by the virus, or to a viral infection after the excision of the SCC. Foulongne et al., on the other hand, detected viral DNA in 71% of non-tumoral skin samples of patients with MCC. However, the MCPyV DNA levels appeared to be higher in MCC tumour tissue than in nontumoral skin in their set of patients [25]. Furthermore, based on the presence of LT-truncated MCPyV in approximately 15% of SCCs from immunocompetents in their patient set, Dworkin et al. [26] even suggested a causative role of MCPyV in SCC, in analogy to Shuda et al. [11] who showed that the LT antigen derived from MCC tumor areas is truncated and lacks the LT MCPyV helicase. This in turn leads to replication incompetence in the virus, thus suggesting an early precancerous integration of the virus into the genome.

Recently, a monoclonal antibody CM2B4 was developed against a peptide fragment of the MCV T antigen exon 2 [27]. The antibody was immunoreactive with the majority of the PCR-positive tumors (77%) [28] and showed also high specificity. Immunohistochemistry as a relatively cheap routine procedure may become the method of detection of MCPyV, but the sensitivity issues will have to be addressed.

In summary, we found MCPyV in 66.6% of our Swiss MCC patient set. This number is similar to the reported incidences in two American MCC patient sets (69% [20] and 80% [8]) and the European experience (85% [18] and 79.8% [13]), but higher than in an Australian patient set (24% [20]). It has been speculated that the differences in the virus prevalence may be due to a higher contribution of UV in the pathogenesis of MCC in Australian patients, or to the presence of virus that remained undetected in the assays which were applied, but not to the quality of DNA used in the assays, as the PCR controls were considered excellent in the Australian set with the low detection rate of MCPyV [20].

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References

1 Toker C: Trabecular carcinoma of the skin. Arch Dermatol 1972;105:107–110.
2 Hodgson NC: Merkel cell carcinoma: changing incidence trends. J Surg Oncol 2005;89:1–4.
3 American Cancer Society: Cancer Facts & Figures 2006. http://www.cancer.org/Research/CancerFactsFigures/cancer-facts-figures-2006.
4 Swann MH, Yoon J: Merkel cell carcinoma. Semin Oncol 2007;34:51–56.
5 Penn I, First MR: Merkel's cell carcinoma in organ recipients: report of 41 cases. Transplantation 1999;68:1717–1721.
6 Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW: Merkel cell carcinoma and HIV infection. Lancet 2002;359:497–498.
7 Rubel JR, Milford EL, Abdi R: Cutaneous neoplasms in renal transplant recipients. Eur J Dermatol 2002;12:532–535.
8 Feng H, Shuda M, Chang Y, Moore PS: Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319:1096–1100.
9 Acott PD, Hirsch HH: Bk virus infection, replication, and diseases in pediatric kidney transplantation. Pediatr Nephrol 2007;22:1243–1250.
10 Caracciolo V, Reiss K, Khalili K, De Falco G, Giordano A: Role of the interaction between large t antigen and rb family members in the oncogenicity of JC virus. Oncogene 2006;25:5294–5301.
11 Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y: T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci USA 2008;105:16272–16277.
12 http://blast.ncbi.nlm.nih.gov/Blast.cgi.
13 Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H: Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst 2009;101:938–945.
14 Lemos B, Nghiem P: Merkel cell carcinoma: more deaths but still no pathway to blame. J Invest Dermatol 2007;127:2100–2103.
15 Varga E, Kiss M, Szabo K, Kemeny L: Detection of Merkel cell polyomavirus DNA in Merkel cell carcinomas. Br J Dermatol 2009;161:930–932.
16 Foulongne V, Kluger N, Dereure O, Brieu N, Guillot B, Segondy M: Merkel cell polyomavirus and Merkel cell carcinoma, France. Emerg Infect Dis 2008;14:1491–1493.
17 Kassem A, Schopflin A, Diaz C, Weyers W, Stickeler E, Werner M, Zur Hausen A: Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the vp1 gene. Cancer Res 2008;68:5009–5013.
18 Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D: MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. J Invest Dermatol 2009;129:248–250.
19 Duncavage EJ, Zehnbauer BA, Pfeifer JD: Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma. Mod Pathol 2009;22:516–521.

20 Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P: Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol 2009; 129:246–248.
21 Wetzels CT, Hoefnagel JG, Bakkers JM, Dijkman HB, Blokx WA, Melchers WJ: Ultrastructural proof of polyomavirus in Merkel cell carcinoma tumour cells and its absence in small cell carcinoma of the lung. PLoS One 2009;4:e4958.
22 Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, Almeida A, Boitier F, Carlotti A, Couturaud B, Dupin N: Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. J Pathol 2009;218:48–56.
23 Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, Stickeler E, Weyers W, Diaz C, Werner M, Nashan D, Zur Hausen A: Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. Int J Cancer 2009;125:356–361.
24 Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ: Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. J Cutan Pathol 2009, Epub ahead of print.
25 Foulongne V, Dereure O, Kluger N, Moles JP, Guillot B, Segondy M: Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases. Br J Dermatol 2010;162:59–63.
26 Dworkin AM, Tseng SY, Allain DC, Iwenofu OH, Peters SB, Toland AE: Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. J Invest Dermatol 2009;129:2868–2874.
27 Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernandez-Figueras MT, Tolstov Y, Gjoerup O, Mansukhani MM, Swerdlow SH, Chaudhary PM, Kirkwood JM, Nalesnik MA, Kant JA, Weiss LM, Moore PS, Chang Y: Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. Int J Cancer 2009;125:1243–1249.
28 Busam KJ, Jungbluth AA, Rekthman N, Coit D, Pulitzer M, Bini J, Arora R, Hanson NC, Tassello JA, Frosina D, Moore P, Chang Y: Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. Am J Surg Pathol 2009; 33:1378–1385.